

ORIGINAL RESEARCH

SERUM NITRIC OXIDE METABOLITES AND ADENOSINE DEAMINASE ACTIVITY IN ASTHMA AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A CROSS-SECTIONAL COMPARATIVE STUDY

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ABSTRACT

Background: Nitric oxide (NO)-related metabolites and adenosine deaminase (ADA) are linked to inflammatory signaling in the respiratory tract, but circulating concentrations may differ between asthma and chronic obstructive pulmonary disease (COPD). **Objective:** To compare serum NO-related metabolites and ADA activity among healthy controls, patients with bronchial asthma, and patients with clinically stable COPD, and to examine their associations with lung function. **Methods:** This cross-sectional comparative study included 220 participants: 100 healthy non-smoking controls, 60 patients with asthma, and 60 patients with COPD. Serum NO-related metabolites were estimated using a kinetic cadmium-reduction method, and serum ADA activity was measured by the Giusti-Galanti colorimetric method. Spirometry included forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and FEV1/FVC. Group differences were assessed using one-way analysis of variance followed by Tukey post hoc comparisons, and associations with FEV1 (% predicted) were examined using Pearson correlation. **Results:** Mean serum NO-related metabolite concentrations were 19.5 ± 4.2 $\mu\text{mol/L}$ in controls, 32.1 ± 5.8 $\mu\text{mol/L}$ in asthma, and 27.4 ± 6.1 $\mu\text{mol/L}$ in COPD (overall $p < 0.001$). Mean ADA activity was 12.3 ± 2.7 U/L, 22.8 ± 4.5 U/L, and 26.1 ± 5.2 U/L, respectively (overall $p < 0.001$). Post hoc testing indicated significant differences between all three groups. In the disease groups, serum NO-related metabolites and ADA activity were inversely correlated with FEV1 (% predicted). **Conclusion:** Asthma and COPD were associated with higher circulating NO-related metabolites and ADA activity than observed in healthy controls, with the highest NO-related metabolite concentration in asthma and the highest ADA activity in COPD. These findings support further investigation of these measures as adjunctive biomarkers of inflammatory burden; however, the cross-sectional design, potential confounding, and use of a systemic NO surrogate preclude causal or diagnostic claims.

Keywords: Adenosine deaminase; Asthma; Chronic obstructive pulmonary disease; Nitric oxide metabolites; Nitrate; spirometry; Oxidative stress

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common chronic respiratory disorders characterized by airway inflammation and impaired airflow, although their clinical course, inflammatory phenotypes, and reversibility of airflow limitation differ substantially. Asthma is characterized by variable respiratory symptoms and variable expiratory airflow limitation and is frequently associated with type 2 airway inflammation, whereas COPD is a heterogeneous condition defined by persistent respiratory symptoms and airflow obstruction arising from airway and/or alveolar abnormalities [1,2].

Oxidative and nitrosative stress contribute to airway injury, inflammatory cell activation, and altered bronchomotor responses in both conditions. In asthma, reactive oxygen species and reactive nitrogen species can amplify epithelial injury and inflammatory signaling [3]. COPD is likewise associated with sustained inflammation involving macrophages, neutrophils, lymphocyte subsets, structural cells, and numerous inflammatory mediators [4].

Nitric oxide (NO) is generated from L-arginine by nitric oxide synthase isoforms and has complex physiological and pathophysiological effects in the respiratory system [5]. Inducible nitric oxide synthase is upregulated during airway inflammation, particularly in type 2 asthma. Exhaled NO is an established airway biomarker in selected clinical settings, but circulating stable NO metabolites represent a biologically distinct systemic measure and should not be considered interchangeable with fractional exhaled NO (FeNO) [5]. Previous studies have demonstrated increased endogenous NO production or NO-derived metabolites in asthma and COPD, with disease-specific differences in magnitude and compartment [6].

Adenosine deaminase (ADA; EC 3.5.4.4) catalyzes the irreversible deamination of adenosine to inosine. By regulating extracellular and intracellular adenosine availability, ADA participates in immune regulation and inflammatory signaling [7]. Adenosine itself exerts context-dependent effects through four G-protein-coupled receptors and is increasingly recognized as an important modulator of inflammation and tissue responses [8]. Altered ADA activity has therefore been investigated in inflammatory and pulmonary disorders, although findings in COPD have not been uniform across populations and disease states.

The present study was designed to compare circulating NO-related metabolites and ADA activity among healthy individuals, patients with bronchial asthma, and patients with COPD, and to examine whether these biochemical measures are associated with spirometric airflow limitation. The original hypothesis was that concurrent alterations in NO-related metabolism and ADA activity may reflect overlapping inflammatory pathways in chronic airway disease; however, the study was observational and did not directly test a mechanistic interaction between these pathways.

MATERIALS AND METHOD

Study design and setting

A cross-sectional comparative study was conducted in the Department of Biochemistry, DVVPF's Medical College, Ahilyanagar, Maharashtra, India, in collaboration with respiratory physicians. Patients were recruited from inpatient and outpatient clinical services. The study protocol received approval from the institutional ethics committee, and written informed consent was obtained from all participants before enrolment.

Study population

A total of 220 participants were included and classified into three groups: 100 healthy controls, 60 patients with bronchial asthma, and 60 patients with COPD. Healthy controls were non-smoking adults aged 18-65 years with no history of respiratory illness. Asthma was diagnosed clinically by respiratory physicians in accordance with the diagnostic framework of the Global Initiative for Asthma (GINA) [1]. COPD was diagnosed by respiratory physicians using clinical assessment and spirometry in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) framework [2]. Patients with acute COPD exacerbations were excluded; the COPD group therefore represented clinically stable disease.

Participants with systemic illness, recent respiratory infection, other chronic inflammatory disorders, congenital abnormalities relevant to the study, or conditions expected to interfere with the biochemical measurements were excluded. Spirometric airflow obstruction was considered in patient selection, including an FEV1/FVC ratio below 0.70. Bronchodilator status for this ratio was not documented.

Biochemical measurements

Serum NO-related metabolites were estimated using the kinetic cadmium-reduction method described by Cortas and Wakid [9]. This assay reduces inorganic nitrate to nitrite, which is then quantified colorimetrically; the measured variable is therefore more accurately interpreted as a stable circulating NO-related metabolite rather than direct serum NO. Results are reported in $\mu\text{mol/L}$.

Serum ADA activity was determined using the colorimetric method of Giusti and Galanti [10]. The assay is based on ADA-catalyzed deamination of adenosine and colorimetric quantification of the ammonia generated, with activity expressed as U/L.

Pulmonary function assessment

Spirometry was performed to record FVC, FEV1, and the FEV1/FVC ratio. FEV1 expressed as percentage of predicted was used for correlation analyses with the biochemical markers. No bronchial provocation testing was reported; accordingly, airway hyperresponsiveness was not treated as a directly measured study outcome.

Statistical analysis

Data were analysed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean $\pm$ standard deviation. Between-group differences in serum NO-related metabolites and ADA activity were assessed by one-way analysis of variance (ANOVA), followed by Tukey post hoc pairwise comparisons. Pearson correlation analysis was used to examine associations between each biomarker and FEV1 (% predicted) within the asthma and COPD groups. A two-sided p value < 0.05 was considered statistically significant. The reporting of this observational study was aligned, where applicable, with the STROBE recommendations [11].

RESULT

Study groups

The analysis included 220 participants: 100 healthy controls, 60 patients with asthma, and 60 patients with COPD.

Serum NO-related metabolites and ADA activity

Both biochemical measures differed significantly among the three study groups (Table 1). Serum NO-related metabolite concentration was highest in the asthma group, intermediate in the COPD group, and lowest in controls (overall p < 0.001). In contrast, serum ADA activity was highest in COPD, followed by asthma, and lowest in controls (overall p < 0.001). Tukey post hoc comparisons showed significant differences between each pair of groups.

Table 1. Serum NO-related metabolites and ADA activity across study groups

Parameter	Control (n=100)	Asthma (n=60)	COPD (n=60)	Overall p value
Serum NO-related metabolites ($\mu\text{mol/L}$)	19.5 $\pm$ 4.2	32.1 $\pm$ 5.8	27.4 $\pm$ 6.1	<0.001
Serum ADA activity (U/L)	12.3 $\pm$ 2.7	22.8 $\pm$ 4.5	26.1 $\pm$ 5.2	<0.001

Values are mean $\pm$ SD. Overall p values are from one-way ANOVA. NO, nitric oxide; ADA, adenosine deaminase.

Table 2. Post hoc pairwise comparisons

Comparison	Serum NO-related metabolites	ADA activity
Control vs asthma	Significant (p <0.001)	Significant (p <0.001)
Control vs COPD	Significant (p <0.001)	Significant (p <0.001)
Asthma vs COPD	p <0.05	p <0.05

Tukey post hoc testing showed statistically significant differences for all pairwise comparisons (p < 0.05).

Association with lung function

Serum NO-related metabolites and ADA activity were inversely correlated with FEV1 (% predicted) in both asthma and COPD (Table 3). The strongest association was between serum NO-related metabolites and FEV1 in asthma (r = -0.62). These findings indicate that higher biomarker concentrations were associated with lower measured lung function within the disease groups.

Table 3. Correlation of biomarkers with FEV1 (% predicted)

Biomarker	Group	Pearson r	p value
Serum NO-related metabolites	Asthma	-0.62	<0.01
Serum NO-related metabolites	COPD	-0.47	<0.05
ADA activity	Asthma	-0.55	<0.01
ADA activity	COPD	-0.51	<0.01

Negative r values indicate that higher biomarker levels were associated with lower FEV1.

DISCUSSION

This study identified distinct but overlapping biochemical patterns in asthma and COPD. Compared with healthy controls, both disease groups had higher circulating NO-related metabolite concentrations and higher ADA activity. Serum NO-related metabolites were highest in asthma, whereas ADA activity was highest in COPD. In both diseases, higher values of each biomarker were associated with lower FEV1 (% predicted).

The higher NO-related metabolite concentration in asthma is biologically plausible in the context of increased inducible nitric oxide synthase activity and nitrosative stress in inflamed airways [5]. Airway NO is especially relevant to type 2 inflammation, and standardized FeNO measurement has established clinical utility in selected asthma populations [12]. However, the present study measured a serum NO-related metabolite rather than FeNO. The distinction is important: serum nitrate reflects systemic NO metabolism and is influenced by biological and pre-analytical factors outside the airways. The present findings therefore support an association with inflammatory disease but should not be interpreted as evidence that serum nitrate is equivalent to an airway-specific NO biomarker.

The intermediate increase in NO-related metabolites observed in COPD is also compatible with previous evidence that endogenous NO production and stable NO derivatives can be increased in COPD, although the magnitude and compartment differ from asthma [6]. COPD inflammation is heterogeneous and can involve neutrophilic, macrophage, lymphocytic, and, in a subset of patients, eosinophilic pathways [4]. This heterogeneity may partly explain why NO-related markers show wider variability in COPD than in classic eosinophilic asthma.

The ADA findings require more cautious interpretation. ADA participates in the control of adenosine concentrations and therefore has potential relevance to immune activation and inflammatory homeostasis [7,8]. In the present dataset, ADA activity was elevated in both diseases and was highest in COPD. Nevertheless, published COPD studies have reported divergent findings. Goodarzi and colleagues observed lower total ADA activity in patients with acute COPD exacerbation than in healthy non-smokers [13]. Patidar et al. likewise reported reduced ADA and ADA isoenzyme activity in serum and cellular compartments as COPD severity increased [14]. More recent work has also reported lower ADA in stable and exacerbated COPD relative to controls [15]. The direction of the current result therefore differs from several published series and should be confirmed in an independent cohort with careful characterization of smoking, disease severity, medication exposure, and ADA isoenzymes.

The inverse correlations between both biomarkers and FEV1 suggest that greater biochemical disturbance accompanies more severe airflow limitation. Such cross-sectional correlations, however, cannot establish temporal sequence or prognostic value. They may also reflect confounding by disease severity, smoking, systemic inflammation, treatment, age, or other factors that were not included in the adjusted analysis.

The concurrent changes in NO-related metabolites and ADA activity are compatible with the broader concept that nitrosative stress and purinergic metabolism participate in chronic airway inflammation. They do not, however, demonstrate a direct NO-ADA signaling axis. No interaction analysis, mechanistic experiment,

adenosine measurement, NOS expression assay, or ADA isoenzyme assessment was performed. The findings are therefore best described as parallel biomarker associations rather than evidence of a causal biochemical pathway.

Clinical implications

The low cost and technical accessibility of colorimetric assays make circulating NO-related metabolites and ADA attractive for exploratory biomarker research. At present, the data do not support their use for diagnosis, screening, prognostication, disease phenotyping, or treatment selection. Before clinical application can be considered, larger prospective studies should evaluate reproducibility, confounding, incremental value beyond established clinical measures, and diagnostic or prognostic performance using appropriate multivariable and receiver-operating-characteristic analyses.

Strengths and limitations

The study compared two major chronic airway diseases with a relatively large healthy control group and evaluated biochemical findings alongside spirometric lung function. Important limitations should nevertheless be recognized. First, the cross-sectional design permits assessment of association but not causality or prognosis. Second, healthy controls were explicitly non-smokers, whereas smoking exposure was described as an underlying factor among patients; the absence of smoking-matched controls is therefore a major potential source of confounding, particularly in COPD. Third, demographic characteristics, body mass index, renal function, diet, medication use, corticosteroid exposure, asthma phenotype, COPD severity, exacerbation history, and other conventional inflammatory markers were not available for adjusted analysis. Fourth, the nitrate-based serum assay is an indirect systemic measure of NO biology and cannot be equated with FeNO or airway NO production. Fifth, an a priori sample-size calculation was not reported, and the available methods did not specify spirometry quality-control procedures or bronchodilator status. Finally, the analysis was unadjusted for potential confounders and did not assess diagnostic discrimination or longitudinal outcomes.

CONCLUSION

Patients with asthma and COPD had higher serum NO-related metabolite concentrations and ADA activity than healthy controls in this cross-sectional study. The highest serum NO-related metabolite concentration was observed in asthma, whereas the highest ADA activity was observed in COPD. Both biomarkers were inversely associated with FEV1 (% predicted), suggesting a relationship with airflow limitation. These findings support further investigation of circulating NO-related metabolites and ADA as research biomarkers of inflammatory burden, but they do not establish a mechanistic NO-ADA signaling axis or clinical utility. Validation in well-characterized, smoking-matched, prospectively recruited cohorts with standardized lung-function testing and multivariable analysis is warranted.

Ethics Statement

Ethics approval and informed consent: The study received approval from the institutional ethics committee, and written informed consent was obtained from all participants before enrolment.

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